

FEEDING EXPERIMENTS ILLUSTRATING THE
IMPORTANCE OF ACCESSORY FACTORS IN
NORMAL DIETARIES. BY F. GOWLAND HOPKINS.

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THE experiments described in this paper confirm the work of others in showing that animals cannot grow when fed upon so-called "synthetic" dietaries consisting of mixtures of pure proteins, fats, carbohydrates, and salts. But they show further that a substance or substances present in normal foodstuffs (e.g. milk) can, when added to the dietary in astonishingly small amount, secure the utilization for growth of the protein and energy contained in such artificial mixtures.

The particular experiments, of which an account is now to be given, were undertaken to put upon a more quantitative basis results which I obtained as far back as 1906-1907¹. Since that time, a fuller realization of the fact that (leaving on one side the influence of the inorganic constituents of dietaries) protein supply and energy supply do not alone secure normal nutrition, has arisen from the extremely interesting recent work upon the etiology of such diseases as beri-beri and scurvy². It is not surprising that much work is now being done in connection with the subject; and since the experimental results given in this paper were obtained, the publications of others have covered part of the ground. In particular I may refer to the work of Stepp³ upon mice, and to the extensive researches of Osborne and Mendel⁴ upon rats. But the observations now to be described differ in some important details from those of the authors quoted. They bring out in

¹ The results of experiments made at this time were summarised in Lectures delivered at Guys Hospital in June 1909. Owing to subsequent ill health these Lectures were never published. The results given in the present paper were communicated to the Biochemical Club in October 1911. See also *Analyst*, xxxi. p. 395. 1906.

² For references see Casimir Funk, *This Journal*, XLIII. p. 395. 1911; also *Journ. of State Medicine*, June, 1912; and Holst, *Journ. of Hygiene*, VII. p. 619. 1907.

³ Stepp. *Bioch. Ztsch.* xxii. p. 452. 1909; and *Ztsch. Biol.* LVII. p. 135. 1911.

⁴ Carnegie Institution, Publication No. 156, Parts I and II. 1911.

particular the marked influence of minute additions of normal food constituents in promoting the nutritive power of synthetic dietaries. Stepp approached the subject on the lines of an attempt to estimate the importance of lipoids in nutrition. He found that food mixtures after extraction with lipid solvents could not maintain life in mice. The total material extracted by the solvents when added to the diet made the food efficient once more; but Stepp was unable to obtain this result by adding any known lipid.

The earlier and greater part of the valuable and critical work of Osborne and Mendel was directed to the question as to whether life could be maintained upon a dietary containing a single individual protein instead of mixtures of proteins such as normal dietaries comprise. Their experiments led them to answer this question in the affirmative. Maintenance is possible so long as the protein stored is one not deficient in individual amino-acid groupings; but as was inevitable, their earlier experiments led these authors to realize the importance of factors other than protein and energy supply, and they found in particular that synthetic dietaries which were capable of maintaining the life of full-grown rats (at least in individual cases), were quite unable, no matter what the protein, to maintain the growth processes in young animals. Of this fact I have long been aware, and I have long known from my own observations that extremely small additions of tissue extracts, etc., were sufficient to induce growth, but until the present set of experiments were undertaken I had obtained no data as to how far the amount of food actually eaten intruded as a factor in the phenomenon. An accurate estimation of the amount of food eaten under the conditions of varied growth forms a special feature in the experiments now to be described.

Osborne and Mendel in their later experiments show that the addition of milk products to the food promoted rapid growth in rats which had remained stationary in weight when on the original artificial dietary. The material added they term "protein free milk." It was prepared by removing as far as possible, by precipitation and coagulation, the proteins from fat-free milk, the fluid being then evaporated at low temperature, and the residue ground to powder. This material was primarily used "to furnish the inorganic elements of the diet," and was added to the artificial dietaries in comparatively large amount (28.2 % of the total food mixture), in which it replaced part of the pure carbohydrate. In my experiments, while the artificial diet consisted of casein, fat, starch, sugar and inorganic

salts, the addendum consisted of milk itself; but this was given in such small quantity that the total solids contained in it amounted to no more than from 1 to 3 or 4% of the whole food eaten. This small addition induced normal growth upon dietaries which without it were incapable even of maintenance. A special feature of my experiments was the rigorous use of controls. In each and every experiment two sets of rats, chosen carefully so as to show correspondence in the weight, sex, and origin of the individuals contained in them, were fed side by side. The sole difference in treatment consisted in the administration of the minute ration of milk to one of the sets compared. In some experiments after the relative rates of growth had been compared for a week or two, the small milk ration was transferred to the set which had been previously fed without it. In all cases the influence of the milk upon growth was so large that it could not have been due to any alteration in the quality of the protein eaten or in its ratio, nor, in my own belief, to the presence of any known milk constituent.

Methods. All my experiments were done on rats. The employment of such small animals for metabolism experiments is sometimes looked upon with a disfavour which is only justified in certain cases. Small animals, while certainly unsuitable for one type of experiment, may be especially well suited for another. When for example data as to nitrogenous equilibrium are to be obtained, accuracy is perhaps difficult to secure in the case of rats. The rapidity of their metabolic processes may be undesirable in connection with certain other enquiries; but this very character in their metabolism makes them especially suitable for other investigations, especially those concerned with growth. That many individuals can be dealt with at once, and the results of experiments treated statistically, is an especially valuable feature of work done with small animals. My experiments were all concerned with young rats at a stage when rapid growth is normal. Their initial weight was mostly from 35 to 50 grams, a stage when weight is normally doubled on an efficient dietary in 20 days or less. The animals, unless otherwise mentioned, were always fed two in a cage, bucks being paired with bucks, and does with does. When the amount of food eaten is to be determined, this grouping has the disadvantage that an average for the two animals must be accepted, but when food is given in excess of the quantity required this is not an important point, and any disadvantages are more than counterbalanced by the fact that quite young rats progress more normally when they have a companion

than when kept singly. Great attention was given to the maintenance of a uniform temperature in the laboratory, this being recorded by a registering thermometer. In particular, a constant temperature for day and night was carefully secured. The rats were kept in round wire cages somewhat similar to those used by Henriques and Hansen and by Mendel and Osborne, though shallower and of wider mesh. They stood in trays filled with sawdust, over which was a layer of filter paper. Rats tend to eat both sawdust and paper, so the cages were raised upon legs four inches high.

Two basal diets were used, differing only in the protein they contained. In the one the protein was Merck's pure casein prepared by Hammersten's method, in the other it was the commercial casein preparation known as "Protene." No "roughage" was provided. With rats it appears to be unnecessary, at any rate in experiments of such duration as mine.

The composition of the dry food mixtures was the following:

	Pure casein mixture	"Protene" mixture
Protein	22 p.c.	21.3 p.c.
Starch	42.0 "	42.0 "
Cane Sugar	21.0 "	21.0 "
Lard	12.4 "	12.4 "
Salts	2.6 "	3.3 "

The salts added were obtained by incinerating the normal laboratory food on which the rats had been kept when not under experiment, and consisted of equal parts of the ash of oats and dog-biscuits. The commercial casein contained 3% of ash, raising the amount of salts in the mixture from 2.6% to 3.3%. The calculated energy value of the pure casein mixture is just over 5 calories per gram, and that of the protein mixture almost exactly 5 calories per gram. A series of estimations in the bomb calorimeter gave a mean value of 4.98 for both mixtures. In general, however, the energy values of the diet were directly determined in the mixtures as they were made up for feeding; the protein and starch not being previously dried. To save space in the protocols only the consumption in calories per 100 grams live weight is given. The figures in the protocols give the daily intake calculated from the food consumption of each short period (usually three days) between successive weighings of the rats. The average daily calorie value of this was divided by the mean weight of the animals during the period and multiplied by 100. The total amount eaten, and the protein eaten, can be calculated with close accuracy if 100 calories are reckoned

to correspond with 20 grams solids in either mixture, and to 4.40 and 4.26 grams of protein in the purer and less pure mixture respectively.

In describing such experiments as those in this paper, it is important to give in detail the method of administering the food. The consumption of an artificial dietary may be a good deal affected by its physical condition when administered, especially when the animals are first put upon it.

The protein, starch, and sugar and salts were mixed dry, and the fat rubbed in by hand, the mixture being worked up until of completely uniform composition. It was given to the animal in earthenware vessels. The day's ration for each pair of rats was weighed out dry, and given the desirable consistency in the following way. Half the ration was mixed with enough water to make it into a thin paste, and the remaining half of the dry material was then added little by little, and well stirred in with a glass rod. The final mixture thus obtained was of such a consistency and character that the rats ate it freely, and almost without any tendency to scatter the food. The amount of food given was always in excess of the consumption. Water was supplied in a special vessel. Before the day's ration was administered to either set of rats, each individual of that set which was to receive milk was put into a separate cage, and the measured quantity of milk given as a separate ration. Only after this was consumed—and its consumption never occupied more than a few minutes—were the rats returned in pairs to the original cages. Both sets were then fed simultaneously with the artificial mixture. The composition of the milk was carefully determined from time to time, and the energy content of the solids was occasionally determined in the calorimeter. This scarcely varied from the value of 4.7 calories per gram.

It was found possible to determine the amount of food eaten with great accuracy. A very small quantity might fall through the bottom of the cage on to the filter paper beneath, but this could always be recovered quantitatively. Absolute accuracy was to be obtained by removing such spilled food at frequent intervals, so as to avoid any soakage with the urine. The amount spilled was never more than a minute fraction of the food eaten, or of that weighed back; and in practice no difficulty was found in dealing with it. At each day's feeding, the food left over from the previous day was carefully removed from the vessel which contained it, the small quantity of spilled food added to it, and the whole dried at low temperature till of constant weight. This weight was deducted from the dry weight of the food

mixture as originally weighed out. During certain periods in each experiment, the fæces were collected in order to determine the energy content, so that data as to the absorption of the food might be obtained. With the cages used by me this collection was easy. The greater part of the fæces fell through the bottom of the cage on to the paper beneath, and as this lay upon sawdust, any urine passed was rapidly soaked up, so that very little contamination of the fæces occurred. No attempt to demarcate the fæces of the experimental period was made, but as they were collected for a week, and under precisely similar conditions in the two sets of rats under comparison, such demarcation seemed unnecessary.

Variability of young rats on a normal diet.

Fifty rats from the stocks which supplied animals for the main experiments, were fed upon bread and milk, and the periods observed during which the individuals doubled their weight. The growth curves of many of them were followed for longer periods; but for the purposes of this paper only the times occupied in the doubling of the initial weight need be given. All the animals were fed under identical conditions. The figures are, of course, not put forward as having any general statistical value. The observations were carried out merely in order to obtain some indication of the variability to be expected in rats from the sources used for other experiments. As will be found in the course of a later discussion, some especial importance is attached in this paper to the initial rate of growth of the experimental rats; hence a measure of normal variability at comparatively early periods during feeding was desired. In order to introduce a factor found in the main experiments,—a change of diet namely at the beginning of the observations—the rats used for the experiment under discussion were transferred from the ordinary stock diet of the laboratory (dog-biscuits and oats) to one of bread and milk. Half the animals used were bucks, and half does. The most striking result of the test was its demonstration of a much greater regularity in the variation of the bucks. The initial weights of the animals fed, while mostly near to the average of 50 grams, showed in certain cases a considerable departure from this (46 to 63 grams). Nevertheless, the periods of weight doubling in the case of bucks departed comparatively little from the mean value. The figures will be found in Protocol A. It will be seen that 5 animals had doubled their initial weight on the 13th, 9 upon the 14th, and 5 upon

the 15th day. Thus in the case of 19 rats out of 25, or 76%, the completion of the weight doubling period occurred within these three consecutive days. Two other rats may be grouped with these, one completing its period on the 11th, and one on the 16th day. Of the remainder two doubled their weight on the 20th, one on the 21st, and one on the 26th day. Over 80% therefore showed periods near to and normally grouped round the mean.

On the other hand the does showed much greater irregularity. Eight animals, or 32%, grew at about the same rate as the bucks, completing their weight doubling between the 13th and the 16th days; 6, or 24%, completed it round about the 20th day; another 5 required about 30 days; and the remaining 4 grew still more slowly; one animal, though showing no signs of ill health, had not doubled its weight by the 56th day.

A longer comparison further brought out the relative slowness in the growth of the females. Thus on the 52nd day of the experiment the mean weight of the bucks was their mean initial weight multiplied by 3.58. In the case of the does the corresponding factor was 2.63.

I was unable to take advantage of the information thus obtained by wholly eliminating the more variable sex from my main experiments, a sufficiency of bucks not being always available. But in all the experiments, every effort was made to give each individual rat in one of the sets compared a representative in the other set, corresponding to it in weight, sex and origin.

Comparison of rats on the artificial dietary alone with others taking a small addendum of milk.

My earliest experiments were made with the commercial casein mixture as the basal diet, neither the protein nor the starch contained in the food being extracted with alcohol. On this food rats always grow to some extent; but their growth is greatly accelerated by a minute addition of milk. Of such experiments only those in which complete estimation of the intake was made appear in this paper. Although less striking than experiments showing complete cessation of growth contrasted with a rate of growth which is normal, they have an interest of their own when the significance of relative intake is under discussion. Later I used Merck's pure casein, and finally employed this in admixture with starch which had been thoroughly extracted with alcohol and with specially purified lard. On such a mixture growth

always ceased after a comparatively short period. Any commercial preparation of casein if very thoroughly extracted with alcohol may take the place of Merck's casein. It will be more convenient to give first the results of these experiments with the purest dietaries.

Exp. I. (Fig. 1 and Protocols I and I(a)¹.) In this experiment 12 young rats were used; all being females of smaller initial weight than the average of the animals in other experiments. Six were upon the pure casein dietary alone, six received in addition 2 c.c. of milk each per diem. In the first half of the period the milk solids amounted to about 3.5 % of the whole food eaten, in the latter half to about 2.5 %. The curves of Fig. 1 show the average weight per rat of the two sets at corresponding periods. In this as in other curves the blackened circles denote the set receiving milk. If the protocol be consulted it will be seen that the behaviour of all the individual animals was almost precisely the same. The set without milk grew slowly till the 13th day when growth ceased. By the 20th day loss of weight was established, and a week later five out of the six rats were dead. Meanwhile the set receiving milk had doubled its weight before the 20th day, showing a normal rate of growth. The question of food consumption is discussed in a later section; but it may be pointed out here that from the 13th to the 20th day the set without milk was consuming some 50 calories per 100 grms. live-weight per day: ample for growth under normal circumstances.

Exp. II. (Fig. 2; Protocol II.) This experiment is the only one given in this paper in which estimations of consumption were not made.

¹ The Protocols will be found at the end of the paper.

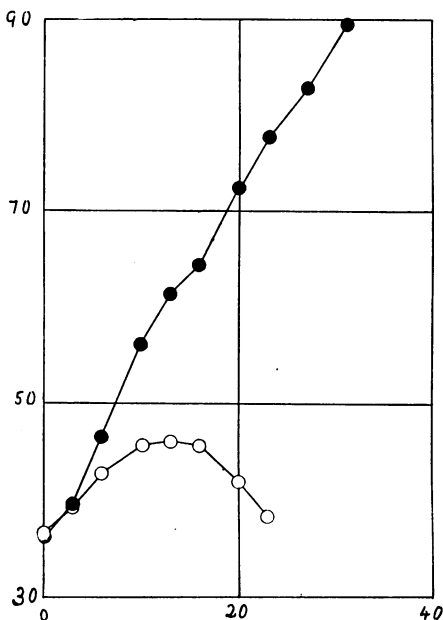


Fig. 1. Lower curve six rats on artificial diet alone. Upper curve six similar animals receiving in addition 2 c.c. of milk each per diem. Abscissæ time in days; ordinates average weight in grms.

The 16 rats used were all bucks, closely agreeing in initial weight, and each set of eight had an identical average weight. The basal diet was a pure casein mixture, all the food being thoroughly extracted with alcohol. One set took this alone, the other animals receiving 3 c.c. of milk each per diem. On the 18th day the milk addendum was transferred from the one set to the other. The striking effect of the milk upon growth is seen in the curves of Fig. 2. Protocol II shows that individual rats agreed closely in their behaviour.

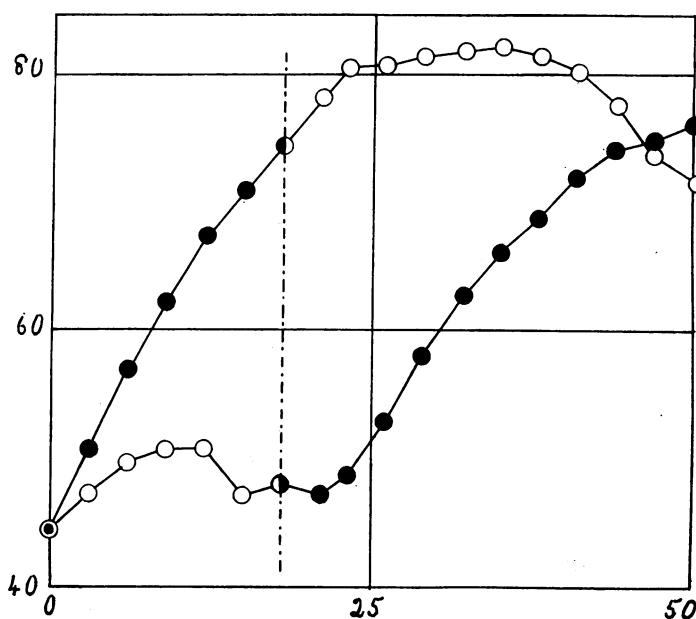


Fig. 2. Lower curve (up to 18th day) eight male rats upon pure dietary; upper curve eight similar rats taking 3 c.c. of milk each a day. On the 18th day, marked by vertical dotted line, the milk was transferred from one set to the other. Average weight in grms. vertical; time in days horizontal.

Exp. III. (Fig. 3; Protocols III (a) and III (b).) This experiment was similar in plan to the last, and similar food was given. Six rats started on the basal diet alone, six received in addition 2 c.c. of milk each per day. On the 19th day the milk ration was transferred from one set to the other. In the first period the milk solids averaged 3.2 % of the food eaten; in the second period just under 4.0 %. At the close of the first period, two of the rats without milk were moribund, and one of those from the milk set died suddenly, though apparently quite

healthy. The second phase of the experiment was therefore carried out with four rats from each set. The average weight of these was somewhat higher than the average of the six in each set at the close of the first period, which accounts for the break in the curves of Fig. 3.

A feature of this experiment was the proportionately large amount of food consumed by all the rats concerned. During the first period the animals without milk were eating as much as 60 calories per 100 grms. of live-weight at a time when their weight ceased to increase. This is much in excess of what is required for growth when the food is normal.

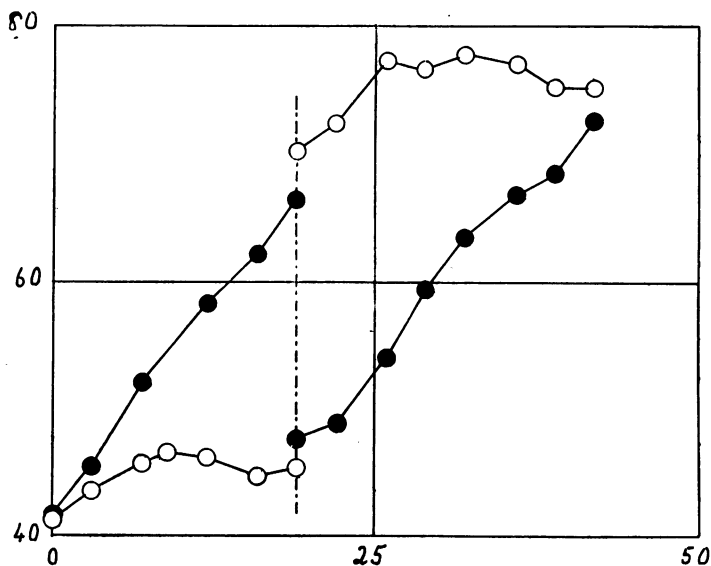


Fig. 3. Curves to the left of the vertical dotted line show average rate of growth of six rats upon the artificial dietary alone (lower curve) and of six receiving 2 c.c. of milk each per diem (upper curve). On the 19th day the conditions were reversed. The second period (to right of dotted line) concerned, however, only four rats out of each set (vide text).

Exp. IV. (Fig. 4; Protocols IV and IV(a).) Of 12 rats used for this experiment, 4 received the pure casein dietary alone. The other 8 were given, for the first 10 days, 1 c.c. of milk each per day, the milk solids amounting to only 1.5 % of the food eaten during this period. This small amount of milk was given to test the lower limits of its power to influence growth. As but little differentiation occurred, the milk was increased on the 10th day, four rats receiving 2 c.c. and four 3 c.c. each per diem. In Fig. 4, the growth curves of three sets are

therefore given; the highest being that of the set receiving the 3 c.c. of milk. I have little doubt from later experience that, had the original dose of 1 c.c. been continued after the 10th day, differentiation from the set without milk would have been plain enough later. The difference in the effect of 2 c.c. and 3 c.c. respectively is seen to be small.

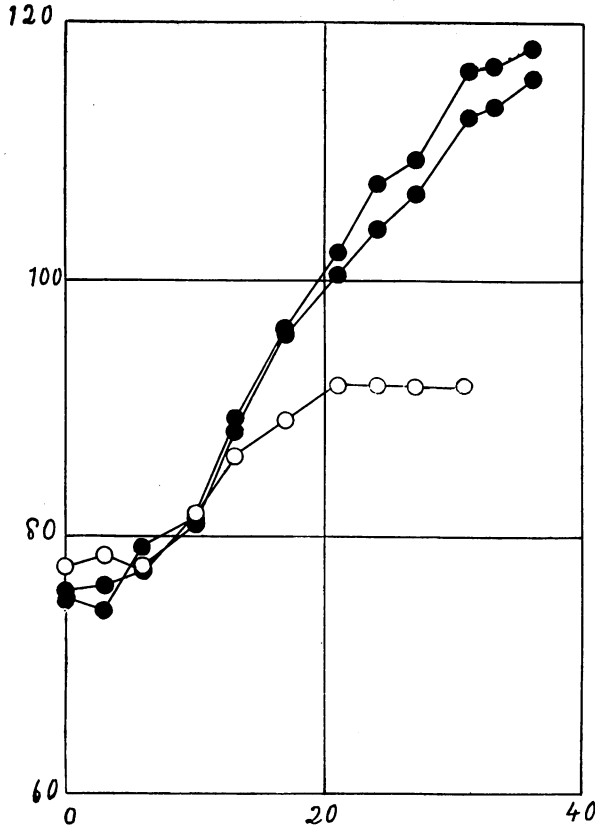


Fig. 4. Lower curve average weights of four rats upon pure casein diet alone. Middle curve average of four rats receiving (from 10th day) 2 c.c. of milk each per diem; upper curve average of four receiving 3 c.c. milk.

Exp. V. (Fig. 5; Protocols V and V(a).) In this experiment the basal dietary was the "protene" mixture. The casein was extracted, but not completely extracted, with alcohol. Eight rats took this mixture alone, and eight received 3 c.c. of milk each per diem. On the 25th day the milk was transferred from one set to the other. By the end of the first period, the rats with milk had increased from an initial total weight of

331 grms. to one of 737 grms. gaining therefore 406 grms. or 120 % of their weight. In the same period those without milk rose from 319.6 grms. to 480.7 grms. showing a gain of only 161 grms. or 50 %. In the second period, the first set, now deprived of the milk ration, soon ceased to grow, and afterwards lost weight. The effect of supplying milk to the other set did not produce so marked a contrast, partly because the acceleration of growth, which was in progress, was momentarily inter-

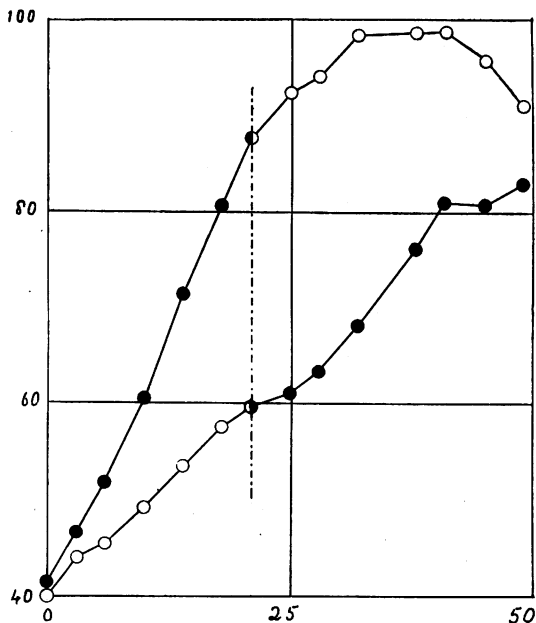


Fig. 5. Eight rats in each set. Lower curve shows growth rate of set starting upon the "protene" diet alone. The set giving upper curve took 3 c.c. of milk each rat per diem. On the 25th day the milk ration was transferred. The vertical dotted line, meant to mark the date of transference, was drawn in error at the 21st instead of the 25th day.

rupted shortly before the close of the experiment by some circumstance which I am unable to explain, but which was certainly accidental (vide Fig. 5). As a matter of fact the transference of the milk was made a little too soon in this experiment. If it had been delayed a few days, the set which was without milk in the first period would, beyond doubt, have ceased to grow, and the effect of the milk made more evident. Nevertheless, as inspection of the curves will show, the effect of the milk addendum and its withdrawal are striking enough. The food consumption is discussed later.

Exp. VI. (Fig. 6; Protocol VI.) In this, the basal food was the "protene" mixture, none of the constituents being extracted with alcohol. Although acceleration of growth in the rats with added milk was sufficiently well marked, the differentiation is less than in the experiments already described. This is in small part due to the fact that the groups compared (unlike those of any other experiment given) were not balanced in respect of sex. The six rats upon the basal diet alone were all bucks, while in each of the two groups of six taking milk, there were two does. But the chief reason for the smaller difference in growth rate was lack of purity in the basal diet. Of the milk rats, one set took $2\frac{1}{2}$ c.c. of milk each a day, the other 5 c.c. After 38 days of feeding, the ratios of initial weight to final weight were as follows:—Without milk 1:2.3; with the smaller amount of milk 1:3.0; with the larger amount of milk 1:3.2. The relative effect of doubling the milk administration is seen to be small. In the curves of Fig. 6, only the first 27 days are given out of 38 to which the experiment extended.

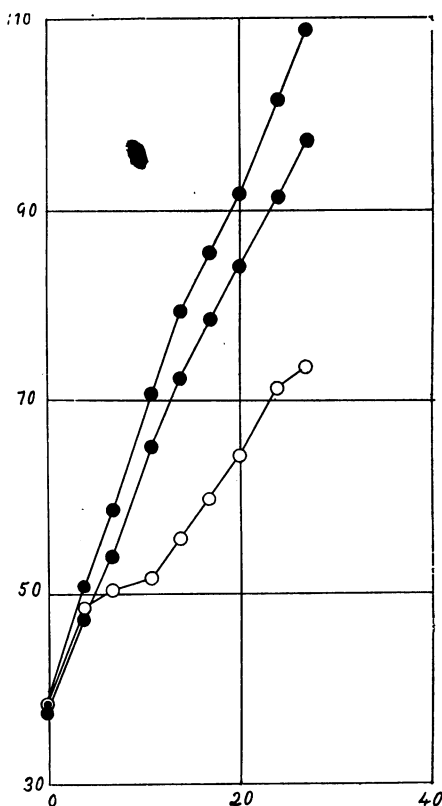


Fig. 6. Eighteen rats in three sets. Lower curve six upon "protene" diet alone; middle curve six taking $2\frac{1}{2}$ c.c. milk per diem; upper curve six taking 5 c.c. milk. Average weights vertical; time in days horizontal. The experiment extended beyond the limits of the curves (vide Protocol VI).

Exp. VII. (Fig. 7; Protocol VII.) This experiment differs from the others in that the initial weights of the rats fed were higher, averaging over 100 grms. It was also of longer duration, the animals being fed for nine weeks. The diet was the "protene" mixture, and none of the constituents were extracted with alcohol. Four rats were

upon the mixture alone, and four received with it 5 c.c. of milk each per day. Four other animals received, instead of the milk, 0.1 gm. of an extract prepared from the expressed juice of mangolds. The observation involving the use of the latter belongs more properly to a

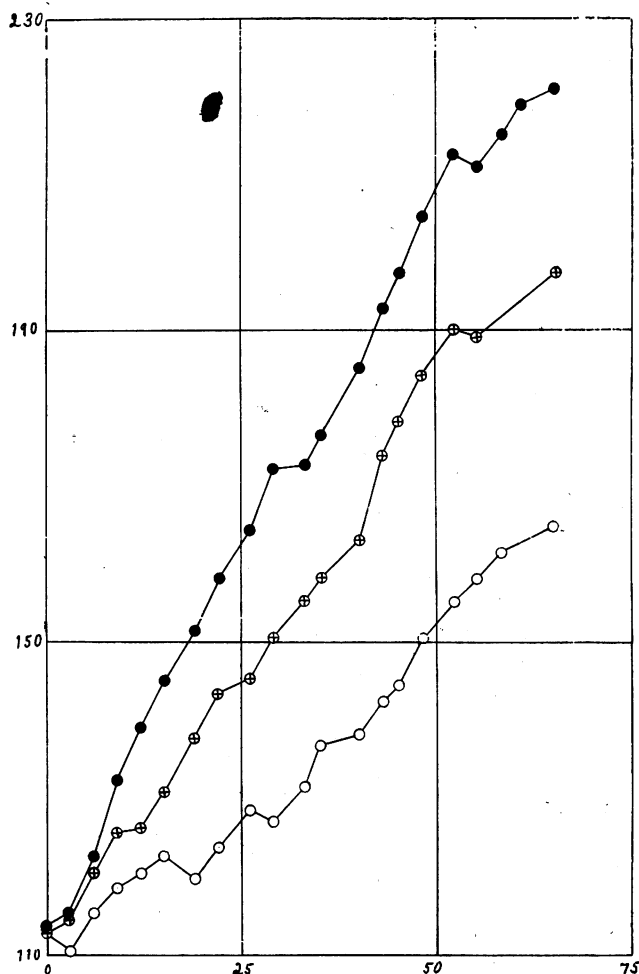


Fig. 7. Lower curve average weights of four rats upon the "protene" diet alone. Middle curve, similar set taking in addition 0.1 gm. of a vegetable extract per rat per diem. Upper curve, similar set taking 5 c.c. of milk as daily addendum to the diet.

research other than that described in this paper. In this case, as in others in which the diet was not fully purified, there was continued slow growth without the milk addendum. The rats on the basal diet

alone appeared indeed in perfect health at the end of nine weeks, and gave no reason to doubt that with continued feeding they would ultimately have reached a normal maximum of body weight, though by so much slower a process than the normal. The growth acceleration induced by the milk addendum (the solids of which, taking the average of the whole period, amounted to 4.2 % of the food eaten) was sufficiently marked. Starting at practically identical weight, the set without milk had on the 25th day gained just 10 % in weight, and those with milk 40 %. At the end of the 61 day period, the respective gains were 44 % and 93 %.

In the above experiments taken together, 42 rats upon the artificial dietary were compared with 52 taking similar food with a small addendum of milk. In every individual case in which the conditions were at all comparable, the rats taking the milk grew much faster than those without it, and the latter, if purity of diet was secured, always ceased to grow altogether after comparatively short periods. In the, as yet unsuccessful, endeavour to isolate individualised substances capable of producing a similar effect upon growth, I have fed altogether a great number of animals, and have seen the failure of artificial food mixtures to support growth in the case of dozens of rats not included in the protocols of this paper. To the question as to whether prolonged maintenance is possible upon synthetic dietaries, my experimental material does not supply a satisfactory answer. Most of the experiments have been of a comparative type, like those here described, not therefore calling for prolonged feeding. But when the protein constituent, and any starch used, have been thoroughly extracted with alcohol, the fat being also purified, I have often found that feeding with an artificial mixture is followed by loss of weight after quite short periods. It is altogether remarkable how small a quantity of associated substances can affect the result.

The milk consumed in the quantitative experiments which have been described, contributed generally some 4 % to the solid matter eaten. It is clear that by far the greater part of the milk solids could play no part in bringing about the result. The minute addition of lactose to the other carbohydrates of the mixture can certainly exact no influence, and if the still more minute addition of lactalbumin to the casein of the basal diet were suspected, or any other effect upon the quality of the protein supply, it is easy to show that it is without significance. The vegetable extract producing the effect seen in Exp. VII was protein free, and I have got equal or greater acceleration

of growth on giving protein-free alcoholic extracts of milk solids, and of yeast, in astonishingly small amounts. If some subtle addendum on the inorganic side be suspected, I may state that an ether extract of yeast (an ether-soluble fraction from the total alcohol extract) practically ash-free, when added in minute amount to the "pure" dietary employed in the above experiments, induced great acceleration of growth. There is some indication from my experiments that the optimum supply of the substances which induce growth is soon reached; but any attempt to ascertain the nature of their action by noting the relation between their concentration and their effects would call for extensive experimentation which it would seem better to leave until definite substances have been isolated.

Special experiments carried out to test the effect of boiling milk indicated that (at any rate if of brief duration) it had no effect upon the capacity to promote growth.

Effect of the milk on absorption from the intestine.

The small ration of milk had certainly no effect on the degree of absorption from the gut. In several of the experiments, as already stated, the energy of the faeces collected over a week was carefully compared with that of the food eaten in the same period. No difference whatever in the actual absorption of the food was found to result from the addition of the milk. The period chosen was in all cases a critical one, in which the animals with milk were growing much more rapidly than those without. The following were the actual data obtained:

TABLE A. *Absorption Coefficients.*

Exp. No.	Period	Calories in food		Calories in faeces		Percent. absorption	
		Rats without milk	Rats with milk	Rats without milk	Rats with milk	Rats without milk	Rats with milk
I	9th to 15th day inclusive	959.0	1512	83.2	125.9	91.3	91.6
III	11th to 17th day inclusive	1042.2	1144.7	96.0	110.7	90.8	90.3
IV	7th to 13th day inclusive	1231.0	1298 (Rats P & Q) 1262 (R & S)	100.9	93.8 109.0	91.8	92.8 91.4

The relation of the rate of growth to the amount of food consumed.

This question is one which involves some points of difficulty. In general, the rapidly growing rats certainly consumed more food (to an extent which will be presently discussed) than those which failed to grow or than those which grew more slowly. It may therefore be suggested that the direct effect of the milk was upon appetite, and that the sequence of events when the milk was added to the synthetic dietary was the obvious one of improved appetite, greater consumption of protein and energy, and consequent increase of growth. It is well to point out, however, that the sequence may be the reverse of the above, and that the acceleration of growth may be the primary, and the increase of appetite a secondary effect.

Appetite may, of course, be markedly affected by the palatability of a ration even in such animals as rats; though with them it is undoubtedly a much less important factor than with animals higher in the scale. But the question of palatability does not intervene in my experiments, which were all comparative. The minute ration of milk which so greatly promoted growth in one of the two sets of rats compared, was, as already stated, given separately in advance of each day's feeding, and after this had been consumed, the animals which had received the milk were fed simultaneously with the control set, and received with them identical food.

In discussions concerning the experimental feeding of animals, the effect of monotony of diet has been given much weight as a factor leading to loss of appetite, and failure of maintenance of growth. Mendel and Osborne have now clearly shown that in the case of rats, this factor has been much over estimated. It cannot, at any rate, affect my experimental comparisons, since monotony was as prominent in the diet of the milk-fed rats as in that of the control animals.

In the case of those rats which continued to grow (whether receiving milk or not) the consumption of food was remarkably steady from day to day. The daily intake slowly increased in absolute amount (though, of course, diminishing relatively to the body-weight) and with slight irregularities continued to do so right to the end of an experiment. Whenever—as in the experiments upon especially pure dietaries—growth ultimately ceased, consumption fell; but as I shall point out immediately, usually not till *after* the cessation of growth was established.

Careful observation of the behaviour of the rats, even towards the end of an experiment, showed that, when first fed each day, the slow growing, or no longer growing set, attacked the food as eagerly as those growing fast. Whenever there was a difference in the amount eaten, it was clearly because satiety was sooner reached by the former, and not from any original distaste¹.

It will, I think, be admitted that the conditions of the experiments described were such that if the small ration of milk affected appetite, it must have been in the special sense of an effect quite indirectly produced. Its influence could not have been exerted upon the lines of increasing the palatability or in diminishing the monotony of the diet.

Schaumann also found that extracts of rice cortex and other substances which, when given to animals upon pathogenic dietaries prevent the onset of beri-beri, also greatly increased the amount of food consumed, although administered separately from the main ration.

Little as we know in detail with regard to the factors (other than æsthetic ones) which control appetite, it is physiologically axiomatic that the rate of metabolism and, in immature animals, the rate of growth are of fundamental importance in determining the amount of food that a healthy animal will in the long run voluntarily consume. What in this sense is a diminution of appetite determines the diminished relative consumption of food observed when a small animal grows larger; the proportionate rate of metabolism and the relative velocity of growth are both diminished in the larger animal, and the consequently lessened demands are, under natural conditions, followed by a smaller relative consumption. Again, while the young animal eats sufficient for growth as well as for maintenance, the adult is content with an intake sufficient for maintenance alone. It may be argued that the voluntary intake is directly affected by the presence or absence of the growth impulse (*Wachstumstrieb*) in the tissues; but it appears more likely to be the actual occurrence of the growth processes, the laying on of new tissue with its own metabolic demands, that affects the instinctive appetite².

If then a factor or factors essential to growth be missing from, or deficient in, a dietary, the consequent arrest of, or diminution in, growth energy may diminish the instinctive consumption of food, while the

¹ The display of abnormal anorexia on the part of individual rats was very rarely observed, and whatever its cause, it was as frequent in the milk-fed rats as in the others.

² Only those perhaps who have had the experience of feeding animals with *excess* of food, and have noted the amount eaten for considerable periods, will realise how well adjusted, under normal circumstances, is the instinctive appetite to the physiological needs.

supply of such factors may increase consumption as an indirect result of a direct affect upon growth.

But, such considerations apart, the question still arises: Was the excess of food eaten by the milk-fed rats proportionate to the great difference in the velocity of their growth and that of the animals fed on the artificial mixture alone?

In those experiments in which the completely purified food-mixture was given, quantitative comparisons are not required in proof of the fact that an agent necessary for growth is absent from the artificial mixture. It will be seen on inspection of the figures that animals fed upon the fully extracted diet may be eating a quantity containing an ample supply both of protein and energy for the continuation of growth at a time when their growth has wholly ceased. It is true that after growth has ceased, or when loss of weight has been established, appetite soon adjusts the intake to a smaller value; but the diminished consumption may be seen to follow and not precede the cessation of growth, and must be, it would seem, the effect and not the cause of it. To make this time-relation clear it is necessary that the intake should be determined for successive short periods, but I have observed it repeatedly in a large number of individual rats.

In Exp. 1 the six rats fed without milk ceased to grow on the 10th day. Yet at this period they were daily consuming food with an energy value of over 50 calories, and a protein content of 2.2 grms. per 100 grms. live-weight (Prot. I (a), Col. 5). This, as all my data show, is more than enough for continued growth in rats of similar size upon a normal diet. Again, in Exp. 4, the consumption of the four animals without milk had similar values, yet they ceased to grow on the 21st day. More striking still are the figures of Exp. 3. The six rats fed upon the artificial dietary without milk grew very little, even from the first, though their energy consumption was high. They began to lose weight on the 9th day; yet on that day, and during the period which immediately followed it, they were consuming over 60 calories per 100 grms. live-weight, and 2.6 grms. of protein (Prot. III (b), Col. 3). These are high values, more than sufficient for rapid growth in rats of their size, when the food has normal characters. The figures given are average values, but if the protocols be consulted it will be seen that the facts fully hold for the individual rats. In the three experiments quoted there were in all sixteen rats upon the artificial dietary without milk. Of these, fifteen showed cessation of growth at a time when their energy and protein consumption were sufficient for normal development.

Such results make it perfectly clear that synthetic diets may wholly fail to support the growth of rats even when consumption is quantitatively quite adequate, and the figures show plainly enough that some factor in diet other than its protein and energy content is indispensable for growth.

When animals, though not growing, are taking food in excess of what is necessary for maintenance and growth, one might think that the supply of a factor which induces growth need only promote the utilization of the food, without increased consumption. Nevertheless in my comparative experiments, in which such a factor was supplied in the small milk ration, the consequent growth was nearly always associated with some degree of increased relative intake; at any rate when the intake is calculated per unit of surface area. Rats growing quickly with milk also consume relatively somewhat more than others growing slowly on the synthetic diet alone. Only a complete metabolic balance sheet therefore can demonstrate to what extent the milk addendum improves the utilization of the food for growth purposes; but I believe that what follows will suggest that the increased intake alone is altogether insufficient to account for the added growth.

It is clear that in comparing the food consumption of two sets of animals which rapidly come to differ in size, we cannot continue to take the absolute consumption for a basis. In the protocols the energy consumption of the rats is given throughout, for convenience sake, in terms of calories per 100 grms. live-weight. It is noteworthy that different sets of rats, whether upon milk or not, if compared for moderately long periods may agree closely in their average consumption of energy when it is thus calculated on a live-weight basis. The correspondence is not seen when one of the sets rapidly fails to grow, as in the experiments with the completely extracted food; but in all the other experiments the rats growing slowly without milk showed remarkable agreement on the above-mentioned lines with those receiving milk and growing rapidly. This is shown by the following figures:

Exp.	No. of rats employed	Period (days)	Average daily consumption in calories per 100 grms. live-weight	
			Without milk	With milk
IV	12	27	49.1	51.5
V	16	21	48.7	49.7
VI	18	24	48.4	50.7
VII	8	21	48.6	47.7

In Exp. 7, the rats which showed the above close agreement when the average consumption was calculated for a period of 21 days, were

fed in all for 61 days. The average intake per 100 grms. per rat when calculated for the whole of this longer period was, of course, lower than the above, but the agreement between the two sets was maintained in spite of their very different rates of growth. The 4 rats taking milk showed an average consumption of 38 calories, and those without it 39 calories per 100 grms. live-weight.

The consumption of food in all cases was entirely dependent upon appetite, and, considering the large number of animals concerned (32 with milk and 22 without) the agreement is not likely to be a coincidence. The slow-growing animals, while necessarily using less than the others for growth purposes, would require relatively more for maintenance, since their mean size for the period was less; but the close adjustment of intake to body-weight is not easy to explain.

It is clear however that, if two sets of animals, rapidly differentiating in respect of body-weight, continue to consume the same amount of food per unit of weight, those which grow faster have available a continuously increasing fraction of the whole for the purposes of growth, since the demands for maintenance continue to become relatively less. For our purpose we need to show what actual proportion of the total intake is available for growth.

If the principles taught by Rubner could be assumed to hold rigidly in the case of animals such as young rats, we might calculate the demands for maintenance at any period during our comparison from the surface areas of the animals concerned, and, deducting this from the total energy consumed, obtain a value for what is available for growth. This could then be compared with the actual rate of growth observed. But the general applicability of the law of surface area has been questioned, and my own experience leads me to believe that at least in the case of very young rats, the demand for maintenance is more nearly determined by the live-weight than by the surface area. It becomes, of course, smaller, relatively to the body-weight as this increases, but it falls off more slowly than the law of surface area would require.

We do not, as a matter of fact, possess at the moment sufficient data to decide what are the maintenance requirements of a young growing rat of a given size, and cannot therefore directly calculate what proportion of its total food consumption is available at any moment for growth.

If the data be examined it will be seen that a given increment of growth is attained with much greater economy when the ration of milk is added to the artificial mixture.

The following table compiled from certain of the experiments gives the food consumption of the rat-sets for definite periods, and the cost of one gram increment in body-weight during such period. Experiments in which the animals on the purer dietary exhibited little or no growth are omitted.

TABLE B. *Relative cost of growth increments.*

Exp.	Length of period	Total calories consumed		Calories required for gain of one grm. body-weight	
		By rats on pure diet alone	By rats receiving milk	Without milk	With milk
I	13 days	1741	2440 (87.9 in milk)	30.4	16.2
IV	27 "	4683	{ 5188 (91.0) 5257 (150) }	75.5	{ 46.3 38.8 }
V	25 "	5024	5701 (225)	35.3	14.2
VI	25 "	4226	{ 5160 (211) 5643 (422) }	22.5	{ 16.0 14.9 }
VII	61 "	13,333	16,782 (686)	64.6	39.9

The absolute values vary greatly in different experiments, the conditions being different. Thus in Exp. 1, the rats were smaller than the average, and only a short period is dealt with. The animals in this experiment were upon the purer food, and those without milk only grew for 13 days. In Exp. 4 the rats were heavier, and being also upon the pure dietary with a very small milk addendum, the figures for them are high. Finally, Exp. 7 is exceptional in that the rats were large, especially at the end of the long period involved, and the cost of a given growth increment is always greater in late than in earlier periods of growth.

But if the cost in energy for unit gain in body weight is compared in each individual experiment, the addition of the milk is seen to involve in all cases a great reduction. A given growth increment is obtained at not much more than half the cost. The energy taken in the form of milk is given in brackets, and is seen to form a very small proportion of the whole consumption. So large an effect upon the relation between food consumption and growth when brought about by so small a variation in the constitution of a dietary forms a sufficiently striking phenomenon; but it is clear that a proof of better utilization (in the direct sense) cannot be based upon it. An acceleration of growth, however accounted for, by diminishing the cost of the maintenance during the acquisition of a given increment in weight, must increase the economy of the growth process. It might still be argued that no matter how the milk ration brings about the increased relative

intake, it is this greater intake which primarily determines the acceleration of growth.

Evidence which appears to refute this view was obtained on the following lines. In each experiment described in this paper, the groups of rats compared were initially of almost exactly equal total weight, and were under exactly similar conditions, except for the presence or absence of the small milk ration. Such strictly comparable animals showed, however, immediate differentiation in their rate of growth. If therefore we note the initial growth velocities and the initial consumption of food, we may make a comparison before differentiation in size has become great enough to introduce complications on the score of maintenance. The necessity arises, it is true, to feed the animals for a week or ten days before any reliable estimations of intake can be made. In order, therefore, to make comparisons at this early period easier, the following empirical data were obtained. Rats similar in

TABLE C. *Effect upon growth of increasing the intake in known proportions.*

45 cals. per 100 grms. live weight			50 cals. per 100 grms. live weight			55 cals. per 100 grms. live weight			60 cals. per 100 grms. live weight		
Initial weight	Wt. on 9th day	Percent. gain	Initial weight	Wt. on 9th day	Percent. gain	Initial weight	Wt. on 9th day	Percent. gain	Initial weight	Wt. on 9th day	Percent. gain
53.3	59.2	11.0	42.1	56.5	34.0	53.0	61.7	16.0	41.2	45.7	13.0
41.0	50.3	23.0	51.3	55.0	7.0	47.7	52.2	9.0	42.0	61.5	46.0
47.5	56.6	19.0	47.0	47.3	0.6	38.5	54.0	40.0	47.8	65.6	37.0
40.0	48.5	20.0	45.7	62.5	37.0	40.0	50.7	27.0	41.0	53.4	30.0
40.0	47.5	19.0	45.0	52.0	16.0	40.0	57.0	43.0	52.0	91.3	37.0
50.5	56.5	13.0	43.4	62.4	44.0	34.5	34.5	0.0	54.2	72.0	35.0
45.5	51.8	14.0	39.5	52.0	32.0	42.0	59.2	41.0	32.5	37.5	15.0
48.0	60.1	25.0	44.7	59.2	32.0	41.0	53.2	30.0	35.5	53.2	50.0
40.5	45.8	12.0	45.9	57.0	24.0	41.0	41.5	1.0	37.8	41.0	13.0
42.2	54.4	23.0	46.0	56.5	23.0	41.2	43.0	5.0	31.2	35.0	12.0
50.2	66.0	31.4	43.0	49.5	15.0	38.2	51.2	34.0	40.0	57.5	44.0
45.2	55.5	23.0	55.5	55.5	0.0	37.7	51.7	37.0	57.5	75.0	31.0
			44.0	57.5	31.0	41.8	59.0	45.0	50.0	64.5	29.0
			40.0	49.2	23.0	40.5	60.0	48.0	43.0	48.0	12.0
						49.7	63.5	28.0	43.0	61.0	42.0
						38.5	56.0	45.0			
						39.5	37.7	1.0			
						53.2	66.2	13.0			
Average initial wt.	45.3			45.2			42.2			43.2	
Average percent. gain	19.4			22.8			26.3			29.7	
Mean probable error of calculated average	1.75			3.6			4.0			3.4	

origin, weight, etc., to those used in the main experiments were fed individually with food of exactly known energy content. The effect upon the initial rate of growth of increasing this in definite proportion was observed. The food chosen was the "protene" mixture, combined, to secure efficiency, with a larger proportion of milk than that given in the main experiments. The milk solids formed 10% of the whole mixture. The composition of the food administered to each group was exactly the same; the amount of protein and other constituents being therefore increased proportionately with the energy. The amounts administered represented 45, 50, 55 and 60 calories per 100 grms. live-weight. These quantities were given on the 1st day; the rats being weighed again on the 3rd and 6th days, and the food then increased so as to give the above values in relation to the increased weight. The consumption was therefore not strictly at the values stated for each day of the period; but the sets so fed are sufficiently comparable both with each other and with the animals of the main experiments. In Table C only those animals are scheduled which wholly consumed the food given. In the case of the highest value (60 calories) a few rats had to be eliminated as not having eaten the whole. When 65 calories per 100 grms. body-weight were administered, only a small proportion of the animals consumed the whole ration. The temperature range during these experiments was from 16° to 19° C., any differences falling equally on all the sets compared. The range was similar in the main experiments.

The data obtained are not wholly satisfactory, firstly because variation in the gain of individual rats within each group is large and irregular, and secondly because for a reason difficult to discover the mean gain of the animals for a given caloric value of intake is distinctly less than that of the milk-fed animals in the main experiments. But the results should, I think, be accepted as giving the information we require, namely, the order of increase in growth velocity which may be expected to occur when the intake is increased without any qualitative alteration in the food. Within the limits studied it is seen that the growth of rats increases but slowly with a rise in intake.

If we compare these results with those of the main experiments, we find that although, in the latter, the initial consumption per unit of live-weight was somewhat greater in those rats which received milk than in those fed without it, the increase in the velocity of growth due to the milk was out of all proportion (if we may judge from the data just given) to what could have been accounted for by the plus in intake

alone. This will be seen on inspection of the following figures from the initial periods of the main experiments.

TABLE D. *Comparison of intake and initial growth-rates.*

Exp.	Period	Average daily intake (calories) per 100 grms. live weight		Percentage excess of intake in milk rats	Mean percentage gain during period		Ratio of growth velocities
		Rats with- out milk	Rats with milk		Without milk	With milk	
I	First 10 days	54.9	59.4	10.0	25.2	54.5	1 : 2.16
III	" 9 "	61.2	62.5	2.1	12.8	35.1	1 : 2.74
IV	" 7 "	55.6	{ 56.4 55.9	{ 1.4 0.5	8.9	{ 17.7 18.6	{ 1 : 2.0 1 : 2.1
V	" 10 "	48.5	50.5	4.1	23.5	46.3	1 : 2.0
VI	" 10 "	50.6	{ 56.1 53.4	{ 10.8 5.2	33.7	{ 73.0 84.2	{ 1 : 2.17 1 : 2.5
VII	" 9 "	49.1	51.3	4.4	9.0	19.0	1 : 2.1

The highest difference in the intake per 100 grms. body-weight, as between the sets with milk and those without, was 10.8 % (one of the sets in Expt. 6). In most cases it was much less than this, and it was sometimes very small indeed. Yet the initial growth velocity was in all cases doubled, and sometimes showed a still greater increase. On the other hand in the experiments of Table C, an increase of 33 % in the intake (from 45 calories to 60 calories) was insufficient to double the rate of growth. An increase of 10 % (*e.g.* from 50 to 55 calories) produced a very small effect. As soon as the intake is high enough to establish growth, the growth velocity increases but slowly with increase of food when the quality remains the same.

Final discussion.

Convinced of the importance of accurate diet factors by my own earlier observations, I ventured, in an address delivered in November 1906, to make the following remarks:

"But, further, no animal can live upon a mixture of pure protein, fat, and carbohydrate, and even when the necessary inorganic material is carefully supplied the animal still cannot flourish. The animal body is adjusted to live either upon plant tissues or the tissues of other animals, and these contain countless substances other than the proteins, carbohydrates, and fats. Physiological evolution, I believe, has made some of these well-nigh as essential as are the basal constituents of diet, lecithin, for instance, has been repeatedly shown to have a marked influence upon nutrition, and this just happens to be something already familiar, and a

substance that happens to have been tried. The field is almost unexplored; only is it certain that there are many minor factors in all diets, of which the body takes account. In diseases such as rickets, and particularly in scurvy, we have had for long years knowledge of a dietetic factor; but though we know how to benefit these conditions empirically, the scale errors in the diet are to this day quite obscure. They are, however, certainly of the kind which comprises these minimal qualitative factors that I am considering. Scurvy and rickets are conditions so severe that they force themselves upon our attention; but many other nutritive errors affect the health of individuals to a degree most important to themselves, and some of them depend upon unsuspected dietetic factors¹."

Evidence has now accumulated from various sides to justify these views. That a deficiency in quite other factors can induce disease is a fact which is now upon a firm experimental basis. That a deficiency, quite as little related to energy supply, may result in the failure of so fundamental a phenomenon as growth in young animals seems equally certain. To what extent bare maintenance of the body-weight is possible, in spite of such deficiencies, is perhaps less certain. Osborne and Mendel observed prolonged maintenance on artificial mixtures, but found that "sooner or later the animals declined; and, unless a change in the diet was now instituted, within a comparatively short period the animals died." I have myself seen quite young rats maintain their weight practically unaltered upon a casein mixture for three weeks, and then begin to lose weight, or on the other hand, if given the necessary small addendum, begin to grow briskly. Such observations give the impression that the factor missing from the artificial food is one concerned solely with growth. But it is certain, as Stepp also found, that the presence of a most extraordinarily small remainder of the substance or substances removable by alcohol extraction, can affect the physiological value of artificial diets; and I am inclined to believe that apparent maintenance (which is usually very slow growth, or very slow decline) is only seen when the diet is not wholly free from them. If the food has been very thoroughly extracted, and if the fat subsequently added is wholly free from any tissue elements, I venture to think that only very short maintenance is possible. That "Denaturierung" plays no part here is shown by the fact that such food is clearly utilised when associated with a small addendum of the kind being discussed.

¹ *Analyst*, xxxi. p. 395. 1906.

If there are any experiments in the literature of nutrition which might be thought to throw doubt upon the importance of such accessory substances, they would seem to be those demonstrating that fully hydrolised proteins can maintain growth. So far as these have been carried out with such material as autodigested pancreas, as in Otto Lœwi's original experiments, or with digested flesh, the mixture would not be necessarily deficient in accessory substances, and inspection of the results seems to show, that when, instead of gland or flesh, a separated protein was used, the effect upon nitrogenous equilibrium or growth was distinctly less favourable. Thus an experiment made by Abderhalden and Rona¹ in which flesh was the source of the digested mixture, showed a much better nutrition balance than a similar one made by Abderhalden and Oppler² who used casein. In Abderhalden's latest experiments of this kind, when digested casein was fed for any length of time, the results were also less favourable than when digested flesh was used³. In any case, as Mendel and Osborne remark, the duration of all such experiments has been too short for deficiencies of the kind under discussion to manifest themselves in any pronounced manner. Thirty days in the case of a slow growing animal such as a dog would be represented by a very few days in the growth period of a rat, and the latter nearly always grows for a brief period after being transferred to an artificial food mixture. A certain store of the missing factors is probably available in the body.

One point bearing on a comparison between Osborne and Mendel's experiments and my own, needs mention here. In the case of four rats these observers state that they found inadequate growth upon an artificial casein mixture, although supplemented by a small milk addendum⁴. The milk was not fresh milk, but "Trumilk" powder, given to the extent of 6 % of the whole food mixture. When later the "protein free milk" (*vide supra*) was added to the extent of 28.2 % of the food, normal growth was established.

It seems to me, however, from inspection of Osborne and Mendel's curves (Nos. L and LI) that in the case of two of these rats, there was no break in the growth curve at the time of the change of food. Normal growth was established upon the casein and Trumilk mixture some ten

¹ *Ztsch. f. physiol. Chem.* LII. p. 507. 1907.

² *Ibid.* LI. p. 226.

³ *Ibid.* LXVII. pp. 50 and 51. 1912. In Exp. V, after a week upon casein products, the nitrogen balance of the dog became negative, though it had been for a long time positive on flesh products.

⁴ *Loc. cit.* p. 104.

days before the change was made. In the case of the other two animals (Charts XLVIII and XLIX) the earlier consumption of food, so far as I can calculate from the food curves given, was certainly for some reason inadequate. Stepp found that milk contained material capable of restoring the nutritive efficiency of extracted diets; but the quantity he used was greatly in excess of that employed by me.

It is possible that what is absent from artificial diets and supplied in such addenda as milk and tissue extracts is of the nature of an organic complex (or of complexes) which the animal body cannot synthesise. But the amount which seems sufficient to secure growth is so small that a catalytic or stimulative function seems more likely. It is probable that our conception of stimulating substances, "Reizstoffe," may have to be extended. The original vague conception of such substances as being condiments, chiefly affecting taste, gained in definiteness by the work of the Pawlov school. But the place of specific diet constituents which stimulate the gastric secretory mechanism can be taken by the products of digestion itself, and in this connection the stimulant in the diet is by no means indispensable. Most observers agree that the addition to normal dietaries of meat extracts capable of stimulating the gastric flow, does not increase the actual absorption of the food, though this point could only be properly tested by adding them to an artificial dietary known to be free from analogous substance. As was emphasized above, the milk did not affect absorption in my experiments. But such undoubted stimulating effects due to diet constituents as those discovered by Pawlov may quite possibly be paralleled elsewhere in the body on more specific and indispensable lines. Stimulation of the internal secretions of the thyroid and pituitary glands, which are believed, on very suggestive evidence, to play an important part in growth processes, can be legitimately thought of. On the other hand the influence upon growing tissues may be direct. If the attachment of such indispensable functions to specific accessory constituents of diets is foreign to current views upon nutrition, so also is the experimental fact that young animals may fail to grow when they are daily absorbing a sufficiency of formative material and energy for the purposes of growth.

In experiments of the kind described great care is required (in respect of cleanliness, maintenance of uniform temperature, regularity in feeding, etc.) in order to keep the animals healthy and rigorously comparable. I am greatly indebted to E. J. Morgan whose industry made the experiments successful.

SUMMARY.

Groups of young rats were fed upon artificial mixtures of isolated casein, fat, carbohydrate, and salts. Side by side with them similar groups were fed on the same basal dietary, but with the addition of a minute ration of fresh milk.

In those experiments in which the basal diet contained fully purified material the rats without milk soon failed to grow. When the constituents were less completely purified, as when the protein was a commercial preparation of casein, slow growth occurred. In all cases the milk addendum, although its total solids amount to only 4 %, or less, of the whole food eaten, induced normal and continued growth. A similar effect was obtained with protein-free and salt-free extracts of milk-solids and of yeast.

The total energy consumption of the animals under comparison was carefully determined, and it could be shown that the rats upon the purer dietary ceased to grow at a time when their intake was more than sufficient quantitatively to maintain normal growth.

The absolute consumption of the animals growing rapidly with the milk addendum was greater than that of those growing slowly (or not growing) upon the basal dietary alone; but the consumption per unit of live-weight, was, in comparable groups, nearly the same.

If growth continued (as upon the less pure basal dietary) it could be shown that the small milk addendum reduced the food consumption necessary for a given weight-increment to one-half or less.

Special experiments were carried out to show the effect upon growth-velocity of known increments in food when its quality is constant.

The results of these showed that any difference in the total intake of the rats in the main experiments, as between those upon milk and others without it, was very much too small to account for the great difference in their growth rates.

The milk ration was fed separately and in advance of the administration of the main dietary. It could not therefore affect the palatability of the food or diminish its monotony. In general, moreover, it was found that cessation of growth upon the pure dietary took place before any failure in appetite, although the consumption might, later, fall to a lower level.

It is therefore suggested that any effect of the addendum upon appetite must have been secondary to a more direct effect upon growth-processes.

Incidentally young rats from the same stocks which were used in the main experiments were fed upon a normal dietary to test their variability. It was found that does exhibited greater variation in growth-rate than bucks, and grew (as others have found) more slowly.

The expenses of this research were in part defrayed by a grant from the Government Grant Committee of the Royal Society.

APPENDIX.

PROTOCOL A. *Variation in growth-rate of rats upon normal diet.*

Rat No.	Initial weight	Time taken to double initial weight, in days	Rat No.	Initial weight	Time taken to double initial weight, in days
Buck 1	63.5	13	Buck 13	46.0	15
„ 2	59.0	14	„ 14	46.0	15
„ 3	52.5	11	„ 15	63.2	26
„ 4	52.2	13	„ 16	56.7	20
„ 5	50.0	15	„ 17	55.3	20
„ 6	47.5	14	„ 18	55.5	15
„ 7	57.0	13	„ 19	55.7	14
„ 8	50.0	14	„ 20	54.0	15
„ 9	60.2	14	„ 21	49.7	16
„ 10	54.0	21	„ 22	49.3	14
„ 11	53.5	14	„ 23	52.0	14
„ 12	54.5	14	„ 24	55.5	13
			„ 25	61.0	13
Doe 1	56.0	13	Doe 13	44.7	16
„ 2	55.5	16	„ 14	53.0	30
„ 3	70.0	16	„ 15	53.0	29
„ 4	58.5	16	„ 16	65.1	56+
„ 5	66.2	21	„ 17	50.4	34
„ 6	61.7	20	„ 18	55.2	34
„ 7	41.6	15	„ 19	49.2	50
„ 8	40.5	13	„ 20	54.8	19
„ 9	51.2	20	„ 21	58.0	26
„ 10	50.2	39	„ 22	45.0	18
„ 11	46.5	41	„ 23	49.5	29
„ 12	44.0	29	„ 24	54.4	22
			„ 25	48.2	16

PROTOCOL I. *Weights and Intake of individual Rats of Exp. I.*

Day No.	Weight of Rat No.		Calcs. per 100 grms. live weight ¹	Weight of Rat No.		Calcs. per 100 grms. live weight ¹	Weight of Rat No.		Calcs. per 100 grms. live weight ¹	Diet, &c.
	A 1♀	A 2♀		B 1♀	B 2♀		C 1♀	C 2♀		
0	37.5	37.0	—	40.0	33.2	—	40.5	30.0	—	Pure dietary
3	39.8	39.3	51.9	45.5	37.0	56.9	43.5	33.0	62.5	+ 2 c.c. milk
6	50.0	42.5	55.0	52.5	41.8	60.9	52.5	41.2	65.5	per rat per
10	59.5	51.4	62.1	64.2	49.0	56.7	63.0	50.0	63.0	diem.
13	67.0	56.4	63.0	69.0	55.2	66.9	68.0	53.0	66.3	The energy
16	69.2	60.0	59.2	73.0	58.0	57.1	71.0	55.5	63.8	of the milk
20	77.5	69.0	54.3	81.0	67.0	55.6	81.0	65.5	60.0	taken is in
23	80.5	76.0	56.9	82.0	71.6	50.1	86.2	70.5	64.0	all cases in-
27	83.5	83.5	55.8	82.0	76.7	58.3	92.6	77.7	68.0	cluded in
31	87.2	91.4	51.4	84.7	79.2	53.1	97.5	84.0	62.5	columns 4,
36	86.5	101.0	44.1	90.5	87.5	58.1	102.2	92.2	60.9	7, and 10.
	D 1♀	D 2♀		E 1♀	E 2♀		F 1♀	F 2♀		
0	44.4	33.2	—	34.5	38.2	—	38.2	31.0	—	Pure dietary
3	47.0	35.2	52.5	38.0	39.9	49.1	40.7	35.1	53.1	alone.
6	52.0	38.2	58.3	41.9	41.8	57.7	46.2	37.2	57.1	
10	59.0	42.0	60.3	44.0	44.5	52.5	47.0	38.4	53.1	
13	59.5	43.4	55.8	46.0	45.0	55.0	44.8	38.0	53.5	
16	58.4	43.0	46.2	45.4	45.5	51.0	44.4	37.5	45.1	
20	53.5	39.5	37.8	42.8	42.7	52.5	40.5	33.2	43.2	
23	50.0	35.0	37.8	39.2	39.2	40.3	39.0	29.7	36.1	
27	40.0	†	—	30.5	33.6	—	35.0	29.0	—	† = death of
31	†	—	—	†	30.5	—	†	†	—	animal.

PROTOCOL I(a). *Average weights, &c. Exp. I.*

Day	Weight of six rats with milk A+B+C	Calories per 100 grms. live-weight (mean)	Weight of six rats without milk D+E+F	Calories per 100 grms. live-weight (mean)	Remarks
0	218.2	—	219.5	—	—
3	238.1	57.1	235.9	51.6	—
6	280.5	60.5	257.3	57.7	—
10	337.1	60.6	274.9	55.3	—
13	368.6	65.4	276.7	54.8	Gain on 13th day:
16	386.7	60.0	274.2	47.4	With milk 150.4 grms. = 68.9 %.
20	441.0	56.6	252.2	44.5	Without milk 57.2 grms. = 26 %.
23	466.8	57.0	230.1	37.7	
27	498.0	60.7	—	—	Five out of six of the
31	526.0	55.7	—	—	rats without milk
36	558.9	54.4	—	—	were dead before the 31st day.

¹ Average daily intake; vide p. 428.

PROTOCOL II. *Exp. II.*

Set A Rat No.	Initial weight	After 18 days with milk	After 32 days with- out milk	Set B Rat No.	Initial weight	After 18 days with- out milk	After 32 days with milk	Remarks
1	44.4	74.0	73.5	9	44.3	47.4	77.5	Pure dietary. For the first
2	45.3	74.5	74.6	10	46.1	48.1	78.3	18 days Set A alone had
3	44.6	73.0	72.0	11	44.7	47.8	77.0	3 c.c. of milk per rat per
4	46.5	77.5	75.0	12	46.9	50.1	78.0	diem. During the last
5	46.2	76.5	72.1	13	46.7	51.0	76.2	32 days Set B received
6	45.0	78.4	70.3	14	44.9	49.2	75.1	the milk, A eating the
7	42.0	72.0	69.3	15	42.5	46.0	73.4	pure diet only.
8	41.2	70.9	70.0	16	40.7	45.2	74.1	All the rats were bucks.
Average weights)	44.4	74.6	72.2		44.6	48.1	76.2	

PROTOCOL III. *Exp. III, First Period.*

Day No.	Weight of Rat No.		Cals. per 100 grms. live-weight per day	Weight of Rat No.		Cals. per 100 grms. live-weight per day	Weight of Rat No.		Cals. per 100 grms. live-weight per day	Diet, &c.
	H 1 ♀	H 2 ♀		I 1 ♀	I 2 ♀		J 1 ♂	J 2 ♂		
0	41.0	40.0	—	44.1	38.5	—	51.7	33.0	—	Pure diet alone.
3	45.2	43.4	69.5	46.1	40.2	66.4	53.5	33.2	63.0	—
7	45.6	47.3	53.5	47.2	41.7	58.0	60.2	33.2	56.5	—
9	44.8	45.5	60.7	48.3	41.7	68.4	62.2	37.2	66.1	—
12	44.5	46.2	64.7	48.5	40.2	58.2	62.2	37.3	60.9	—
16	40.0	42.2	57.7	48.8	36.0	56.7	63.2	38.7	50.2	—
19	38.0	42.5	46.8	49.5	35.8	49.3	65.5	40.2	43.0	At end of period rat No. H 1 was mori- bund & I 2 was ill.
	K 1 ♀	K 2 ♀		L 1 ♀	L 2 ♀		M 1 ♂	M 2 ♂		
0	41.0	40.0	—	43.4	39.2	—	51.1	33.1	—	Pure diet + 2 c.c.
3	45.2	43.8	69.7	49.3	42.2	70.5	56.2	35.7	67.6	milk per rat per
7	52.8	50.8	62.4	57.5	50.0	66.3	65.2	36.6	51.8	diem.
9	57.1	53.8	73.9	62.0	51.7	73.2	71.5	41.5	67.2	—
12	59.0	57.5	64.8	63.5	59.2	67.6	70.5	46.2	59.0	—
16	60.5	59.0	56.0	65.8	63.0	63.8	77.0	52.5	47.2	—
19	63.5	63.5	49.1	66.6	67.5	74.1	86.1	53.3	47.2	On the 19th day rat No. L 1 died from cause unknown.

PROTOCOL III (a). *Exp. III, Second Period.*

Day	Weight of Rat No.		Calories	Weight of Rat No.		Calories	Weight of Rat No.		Calories	Weight of Rat No.		Calories
	I 1	I 2		J 1	J 2		K 1	K 2		L 2	M 1	
19	49.5	35.8	—	65.5	40.2	—	63.5	63.5	—	67.5	86.1	—
22	50.0	37.8	54.2	65.5	42.8	49.1	66.2	66.7	47.5	69.7	87.7	45.2
26	53.1	42.0	55.0	72.1	49.0	51.4	70.5	73.0	52.5	74.0	92.7	51.2
29	57.5	47.2	48.5	77.5	56.0	51.2	70.8	70.0	38.8	74.7	92.0	43.5
32	58.3	51.0	52.7	83.0	62.2	44.9	71.5	71.2	42.2	75.5	93.7	33.4
36	60.5	57.0	44.9	83.0	67.5	40.9	69.7	70.7	42.2	74.0	94.0	39.1
39	63.7	60.5	49.8	86.0	64.2	39.3	67.2	70.0	36.5	74.6	89.5	33.6
42	67.0	66.2	54.2	91.0	66.5	42.2	70.2	72.8	45.8	75.0	83.0	31.1

PROTOCOL III (b). *Average Weights, &c. Exp. III.*

Day	Average weight of rats H, I, J	Average consumption in cala. per 100 grms.	Average weight of K, L, M	Average consumption in cala. per 100 grms.	Diet, &c.
0	41.3	—	41.6	—	H, I, J, (six rats) on pure diet alone.
3	43.6	66.3	45.4	72.8	
7	45.8	56.0	52.1	60.3	K, L, M, (six rats) on pure diet with milk added.
9	46.6	65.1	56.2	50.4	
12	46.2	61.3	58.3	63.8	
16	44.8	54.8	62.2	55.6	
19	45.2	46.4	66.6	56.9	
Average weight of I and J					
Average weight of K+L2 & M1					
19	47.8	—	70.1	—	In second period milk reversed. Four rats in each set.
22	49.0	51.7	72.6	46.2	
26	54.1	53.1	77.5	51.9	
29	59.5	49.8	76.9	41.2	
32	63.6	48.7	77.9	37.8	
36	67.0	42.8	77.1	40.5	
39	68.6	44.5	75.3	35.0	
42	72.6	48.1	75.2	38.4	

PROTOCOL IV. *Exp. IV.*

Day No.	Weight of Rat No.		Cals. per 100 grms. live-weight	Weight of Rat No.		Cals. per 100 grms. live-weight	Weight of Rat No.		Cals. per 100 grms. live-weight	Diet, &c.
	N1♂	N2♂		O1♀	O2♀		P1♂	P2♂		
0	68.6	69.8	—	92.7	79.2	—	59.5	75.5	—	N and O on pure diet alone.
3	72.5	67.7	52.9	94.0	80.7	45.1	58.0	75.5	50.4	P, Q, R, & S on pure diet +
6	72.0	67.5	56.2	96.0	78.7	42.7	62.0	78.0	63.3	milk.
10	75.3	72.2	60.0	95.3	74.4	49.1	61.4	81.6	61.8	Up to 10th day 1 c.c. of milk
13	78.5	80.7	62.6	99.5	78.7	55.6	69.8	88.9	70.5	per rat.
17	84.2	84.7	54.1	102.5	84.9	50.8	78.2	90.5	54.1	After 10th day P & Q received
21	89.1	86.2	54.1	106.4	86.0	52.5	82.3	93.5	61.1	2 cc. and R & S 3 c.c. of
24	85.7	83.7	54.2	107.9	87.0	48.5	86.8	93.0	55.9	milk per rat per diem.
27	85.6	84.8	47.3	107.9	87.1	35.7	91.0	95.0	54.1	On 31st day N 2 was mori-
31	85.0	85.2	—	107.9	87.3	—	95.6	99.0	—	bund.
	Q1♀	Q2♀	Q1+Q2	R1♂	R2♂	R1+R2	S1♀	S2♀	S1+S2	
0	75.0	93.5	—	69.2	62.7	—	93.8	75.5	—	
3	77.7	93.5	45.1	68.4	61.0	45.3	90.5	77.0	49.7	
6	77.7	98.5	52.5	72.0	64.2	47.4	93.2	84.2	54.0	
10	80.5	102.5	46.8	75.2	64.2	58.8	94.5	90.0	54.0	
13	89.7	108.3	56.4	82.7	68.2	61.5	102.0	99.7	55.3	
17	100.2	114.0	49.1	90.0	78.5	59.7	110.0	106.0	47.4	
21	110.9	115.5	53.8	93.5	85.0	63.0	114.4	116.0	59.9	
24	119.0	117.0	45.9	98.7	88.1	55.6	120.5	122.6	52.9	
27	124.2	117.0	38.6	99.4	89.7	41.4	123.7	124.8	45.0	
31	131.8	125.0	—	105.0	92.8	—	132.0	134.8	—	

PROTOCOL IV (a). *Averages Exp. IV.*

Day	Average weight of N and O	Average cals. per 100 grms. live weight	Average weight of P and Q	Average cals. per 100 grms. live weight	Average weight of R and S	Average cals. per 100 grms. live weight
0	77.8	—	75.9	—	75.3	—
3	78.7	49.0	76.2	47.7	74.2	47.5
6	77.3	49.5	77.8	57.8	79.2	50.7
10	81.8	54.6	81.4	54.3	81.0	56.2
13	86.2	59.1	89.2	63.4	88.1	57.9
17	89.1	52.5	95.8	51.6	96.1	53.5
21	91.9	53.2	100.5	57.4	102.2	61.4
24	91.8	51.3	104.0	50.9	107.5	54.2
27	91.7	41.4	106.8	46.4	109.4	43.2
31	91.8	—	112.7	—	116.1	—

PROTOCOL V. *Exp. V.*

Day	Weight of Rat No.		Cals. per 100 grms. live-weight "Protene"	Weight of Rat No.		Cals. per 100 grms. live-weight "Protene"	Weight of Rat No.		Cals. per 100 grms. live-weight "Protene"	Weight of Rat No.		Cals. per 100 grms. live-weight "Protene"
	V 1 ♂	V 2 ♂		V 3 ♀	V 4 ♀		V 5 ♀	V 6 ♀		V 7 ♂	V 8 ♂	
	Without milk		diet	Without milk		diet	Without milk		diet	Without milk		diet
0	35.7	46.0	—	36.7	43.1	—	37.1	37.9	—	38.4	44.7	—
3	39.2	52.0	48.6	40.0	46.4	38.0	41.0	42.7	51.0	42.0	48.5	36.6
6	42.0	54.7	49.2	41.4	48.0	48.4	43.5	43.6	51.2	39.5	51.0	41.2
10	47.6	60.7	52.8	44.7	48.6	51.6	47.4	45.0	55.6	45.3	55.4	57.8
14	52.4	64.2	54.6	48.4	56.8	56.4	50.0	48.2	56.2	49.3	58.7	52.0
18	59.5	70.5	52.2	50.7	59.5	50.6	54.0	53.4	53.6	51.2	61.0	47.2
21	61.7	73.4	45.6	53.0	64.0	46.8	55.0	57.5	44.0	51.0	61.0	35.6
25	66.0	78.0	42.6	54.0	66.0	41.2	54.5	59.2	43.0	47.0	56.0	32.0
	Milk (3 c.c.) for rest of period			Milk (3 c.c.) for rest of period			Milk (3 c.c.) for rest of period			Milk (3 c.c.) for rest of period		
28	72.2	84.2	48.4	57.2	69.0	43.0	58.4	64.5	39.8	45.0	55.0	34.4
32	81.0	93.0	47.6	62.7	74.2	47.6	61.5	72.0	49.6	44.0	55.0	41.4
38	92.0	105.0	46.8	72.5	84.5	50.4	69.0	80.5	46.8	45.7	59.0	45.4
41	95.2	110.0	48.4	77.5	88.0	49.6	73.2	81.0	46.0	47.5	62.0	42.6
45	95.0	109.7	50.6	79.8	89.2	48.0	76.3	82.7	51.6	50.8	62.0	62.6
49	99.7	113.6	47.4	78.2	88.2	47.0	78.9	85.7	52.2	53.5	65.5	60.4
	With milk (3 c.c.)			With milk (3 c.c.)			With milk (3 c.c.)			With milk (3 c.c.)		
0	37.1	47.0	—	38.1	42.0	—	39.4	42.0	—	39.7	45.6	—
3	41.5	54.0	50.4	44.5	48.6	42.6	42.0	47.0	46.8	43.0	52.2	45.6
6	46.0	63.2	50.8	50.0	55.2	48.4	46.6	50.5	52.0	47.5	55.0	47.8
10	49.3	78.6	49.0	60.7	67.4	56.6	52.1	57.5	53.6	54.9	63.8	51.6
14	57.5	91.9	51.8	61.2	69.5	58.8	71.4	76.9	52.6	64.4	77.0	54.4
18	65.5	108.0	49.2	67.0	81.0	50.8	78.2	85.0	49.2	73.4	87.0	49.8
21	70.8	117.5	44.0	72.0	85.0	44.2	85.0	89.0	40.0	88.6	90.0	42.4
25	78.2	130.0	42.0	77.5	91.6	42.0	89.0	93.5	42.8	85.2	92.0	42.6
	Milk stopped on 25th day			Milk stopped on 25th day			Milk stopped on 25th day			Milk stopped on 25th day		
28	81.0	134.5	40.6	77.5	96.2	46.0	90.7	95.7	45.4	87.5	89.5	44.0
32	82.8	140.7	40.6	80.2	100.5	44.8	94.3	102.0	47.0	90.0	94.0	44.0
38	84.2	135.5	37.0	82.5	96.6	46.8	97.0	107.0	44.4	93.4	92.0	44.2
41	83.2	131.2	31.2	82.2	105.5	51.2	100.7	106.2	45.6	92.0	88.0	42.0
45	77.3	129.1	36.4	80.2	102.8	40.8	100.2	103.2	43.6	88.0	85.9	40.0
49	70.1	123.5	46.2	70.2	93.0	47.6	100.0	99.4	43.6	85.2	85.9	45.4

PROTOCOL VI. *Exp. VI.*

Day	Weight of Rat No.		Average intake in cal. per 100 grms. live-wt.	Weight of Rat No.		Average intake in cal. per 100 grms. live-wt.	Weight of Rat No.		Average intake in cal. per 100 grms. live-wt.	Diet, &c. 1
	VI1♂	VI2♂		VI3♂	VI4♂		VI5♂	VI6♂		
0	39.0	35.5	—	40.7	34.0	—	43.0	39.0	—	VI 1 to VI 6 on "Protene" diet alone.
4	49.0	47.5	66.9	50.0	43.5	61.9	52.5	48.0	62.8	
7	48.2	47.9	41.3	53.2	42.5	44.0	58.5	51.0	55.8	
11	55.0	54.0	45.5	54.0	32.7	34.5	63.5	49.7	42.6	
14	63.5	61.5	59.6	61.0	34.5	51.5	63.2	52.0	41.7	
17	69.5	69.5	52.4	64.2	40.2	50.0	64.3	52.0	38.1	
20	75.2	76.0	45.2	69.0	44.2	44.1	69.5	53.2	37.1	
24	82.7	83.0	50.7	72.5	50.0	43.0	78.7	61.7	46.9	
27	90.0	85.5	52.4	75.0	52.2	42.4	77.2	67.5	44.3	
30	95.0	92.5	53.0	77.5	55.2	41.6	83.4	71.2	41.8	
34	104.8	104.5	52.7	80.6	61.7	45.4	86.8	74.6	42.9	
38	113.2	111.3	50.0	83.6	66.4	50.0	89.5	80.6	44.0	
	VI7♂	VI8♂		VI9♂	VI10♂		VI11♀	VI12♀		VI7—VI12 "Pro- tene" diet + 2.5 c.c. milk per rat per diem.
0	34.5	39.0	—	41.0	31.5	—	41.0	39.3	—	
4	42.0	47.2	58.2	50.5	39.5	61.3	51.7	51.5	67.5	
7	47.0	53.0	56.2	56.2	46.0	64.3	61.0	60.0	53.0	
11	57.5	67.5	46.0	65.2	57.0	45.9	73.7	70.0	53.0	
14	64.0	74.5	54.5	76.0	57.0	52.4	83.7	80.0	56.3	
17	66.0	81.0	46.1	81.2	64.5	46.8	92.0	88.5	53.5	
20	72.4	84.5	40.0	86.5	73.0	40.2	95.0	93.5	38.8	
24	80.0	96.5	46.0	97.3	85.4	40.7	96.9	93.0	37.3	
27	87.0	104.2	38.0	103.0	97.0	42.7	100.0	93.0	36.5	
30	93.2	109.2	39.0	110.5	103.0	38.6	104.2	94.0	36.2	
34	104.9	122.2	37.9	118.3	117.7	37.8	111.6	98.3	39.0	
38	114.0	135.0	37.4	130.5	128.4	38.3	115.5	100.2	39.0	
	VI13♂	VI14♂		VI15♂	VI16♂		VI17♀	VI18♀		VI 13—VI 18 "Protene" diet + 5 c.c. milk.
0	41.0	36.5	—	36.7	36.8	—	39.0	42.4	—	
4	48.0	52.0	57.9	45.0	50.0	60.8	53.1	57.0	58.2	
7	59.0	55.8	53.0	52.8	59.5	54.1	62.0	63.7	54.3	
11	74.2	69.7	44.5	61.2	69.0	47.9	75.7	75.7	50.0	
14	86.0	78.5	51.2	68.5	76.0	58.2	83.2	85.1	53.5	
17	95.0	87.0	44.8	70.2	82.2	53.5	88.7	90.5	49.0	
20	101.0	97.0	46.8	75.5	88.7	56.5	93.0	96.4	37.7	
24	114.5	112.5	46.0	82.9	99.4	56.6	98.1	103.5	45.5	
27	125.0	118.2	42.6	91.4	111.0	45.9	100.0	108.4	42.6	
30	135.0	130.5	38.0	101.0	119.5	42.6	100.0	115.0	34.6	
34	140.1	135.1	38.0	114.0	130.2	38.4	102.5	117.8	48.2	
42	150.0	145.2	38.5	117.0	141.0	41.3	100.5	118.2	36.2	

PROTOCOL VI (a). *Averages, Exp. VI.*

Day	Average weight VII-VI6	Average intake in calories per 100 grms. body-weight	Average weight VI7-VI12	Average intake in calories per 100 grms. body-weight	Average weight VI13-VI18	Average intake in calories per 100 grms. body-weight
0	38.5	—	37.7	—	38.5	—
4	48.4	63.9	47.1	62.3	50.8	59.0
7	50.2	47.1	53.9	57.8	58.8	53.8
11	51.5	40.9	65.2	48.3	70.9	47.5
14	55.9	50.9	72.5	54.4	79.5	54.3
17	59.9	46.8	78.9	48.8	85.6	49.1
20	64.5	42.1	84.2	39.7	91.9	47.0
24	71.4	46.9	91.5	41.3	101.8	49.7
27	73.9	46.4	97.3	39.1	109.0	43.7
30	79.1	45.5	102.3	37.9	117.0	38.4
34	85.5	47.0	112.2	38.2	123.3	41.5

PROTOCOL VII. *Exp. VII.*

Day	Weight of Rat No.		Daily intake in calories per 100 grms. live-weight "Protene" mix- ture alone	Weight of Rat No.		Daily intake in calories per 100 grms. live-weight "Protene" mix- ture alone	Weight of Rat No.		Daily intake in calories per 100 grms. live-weight "Protene" mix- ture + 5 c.c. milk per rat	Weight of Rat No.		Daily intake in calories per 100 grms. live-weight "Protene" mix- ture + 5 c.c. milk per rat
	VII1♂	VII2♂		VII3♂	VII4♂		VII5♂	VII6♂		VII7♂	VII8♂	
0	81.5	103.5	—	139.0	130.7	—	98.7	111.0	—	109.2	137.0	—
3	72.5	105.5	48.2	130.7	133.4	48.4	96.0	118.7	—	106.5	139.7	—
6	73.5	109.0	45.2	140.0	139.0	46.8	101.0	129.2	58.5	104.2	157.0	47.3
9	73.5	112.2	48.4	145.2	144.5	45.2	111.0	141.7	54.6	111.0	165.4	43.0
12	79.0	114.5	53.0	146.7	141.7	55.6	117.0	149.2	52.6	120.5	170.0	52.3
15	82.0	119.8	51.5	146.0	143.0	46.8	120.0	151.5	49.0	131.5	177.2	42.3
19	77.2	114.5	46.2	146.5	141.5	46.8	124.5	155.7	46.2	135.7	190.5	44.5
22	84.5	124.5	39.3	147.4	139.5	45.2	127.0	162.0	44.6	144.2	200.0	37.7
26	85.0	127.2	39.0	155.5	146.7	39.0	147.0	166.2	49.0	150.5	214.0	35.9
29	86.5	121.2	40.5	153.0	148.3	40.2	145.7	175.0	45.4	152.5	215.5	37.9
33	89.5	127.5	36.8	156.0	155.0	43.7	146.0	178.0	46.2	154.5	213.0	37.2
35	91.5	132.0	35.9	165.0	159.0	42.1	149.5	181.0	45.2	160.0	216.5	35.2
40	94.5	134.5	32.1	164.0	160.5	46.8	157.0	191.0	36.7	164.7	228.0	34.1
43	97.0	135.0	34.5	172.9	165.0	46.8	165.5	202.2	45.1	169.5	234.2	40.4
45	97.5	136.0	36.3	177.0	168.0	46.8	168.9	206.0	42.6	174.0	240.0	39.3
48	103.7	141.0	38.5	185.0	172.2	40.0	171.7	208.7	37.6	183.5	254.5	31.3
52	107.0	141.5	37.7	191.0	181.0	34.6	179.0	219.5	28.0	188.0	266.0	30.3
55	111.0	145.1	39.1	192.7	184.0	32.8	175.2	217.5	30.4	189.0	264.5	28.4
58	117.0	145.1	38.5	198.0	185.7	29.9	180.2	223.7	30.6	194.0	262.0	24.8
61	120.5	150.0	35.2	200.5	189.2	32.8	182.4	229.2	33.5	197.0	268.5	28.7